Feature

Virtual Neanderthals

Our closest hominid relatives may have died out 30,000 years before the arrival of the computer, but thanks to modern genomics and scanning technology, they are now very present in the 21st century and can even help us understand our own species. **Michael Gross** reports.

In June 2000, the first draft genome sequence of a hominid species, *Homo sapiens*, was presented to a duly impressed public. Only ten years later, the second species followed, *Homo neanderthalensis*. Of the former species, there were more than six billion specimens populating the planet, so the researchers had a practically unlimited supply of DNA. Of the Neanderthals, there are none, and Svante Pääbo's group at the Max-Planck Institute for Evolutionary Anthropology at Leipzig, Germany, had to make do with samples of less than half a gram of powdered bone retrieved using a sterile dentist's drill.

What a difference ten years make. "Ten years ago we were limited to using PCR to retrieve short pieces of DNA from ancient bones found at archaeological excavations. Mostly we were limited to studying the mitochondrial genome because cells contain more copies of the mitochondrial than the nuclear genome so more copies of it are likely to survive," Pääbo explains. "This all changed with high-throughput sequencing techniques. It became possible to sequence all the DNA extracted from a fossil, and to use DNA capture techniques to pull out certain parts of the genome that were of interest."

Along with dozens of contemporary individuals from James Watson to Desmond Tutu, who had their personal genomes sequenced in recent years, *Homo neanderthalensis* reaped the benefits of the enormous leap forward brought by second generation sequencing technology. Neither the microscopic sample size nor the fact that the ancient DNA was shattered into small fragments posed a significant problem for the new generation of sequencing machines. The main challenge, however, was the ubiquitous contamination from a very similar DNA, namely our own.

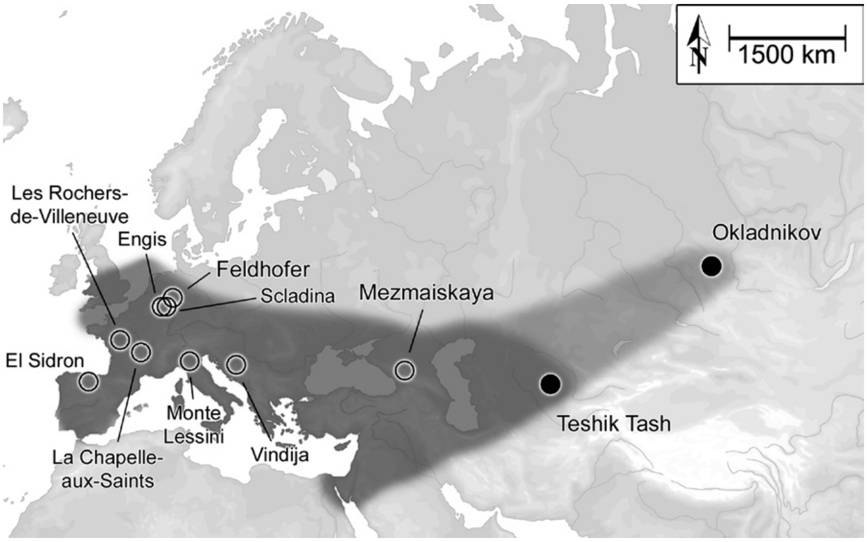
In a 2006 paper marking the first one million bases of the nuclear Neanderthal genome, Pääbo's group expressed the hope that they would finish the entire sequence by 2008. However, the problem of contamination with modern human DNA turned out to be more serious than expected, such that the researchers had to improve their methodology and start over again. The draft sequence with 1.3 fold coverage, based on three separate Neanderthal females, was published in May 2010 (Science (2010), *328*, 710–722), neatly corresponding to the status of the *Homo sapiens* genome exactly 10 years earlier.

What makes us human

While still far from perfect or complete, the Neanderthal data available at that point were good enough to start evolutionary studies based on individual genomes of modern humans from various parts of the globe, using the chimpanzee genome as an external reference point. Thus, gene variants that agree between Neanderthal and chimpanzee, but diverge in modern humans are likely to be specific to us.

The question that attracted the most media attention concerns the population history in the period when our ancestors co-existed with Neanderthals in Asia and Europe. Did Cro-Magnon cavemen and women meet their Neanderthal

Neanderthal life: A Neanderthal hunter using a stone tool to carve up his prey, as shown in an exhibit at the Neanderthal Museum close to the quarry site where the bones of the first Neanderthal described as a distinct species were discovered in 1856. (Photo: copyright Neanderthal Museum/M.Pietrek (http://www.neanderthal.de).)



Old Europe: The map shows the spread of Neanderthal populations in Europe and Asia, with the sites where remains have been found. (Reproduced with permission from Krause *et al.* (2007) Nature *449*, 902–904.)

contemporaries, and did they interbreed? This question is difficult to investigate, as both genotypes are so similar, but Pääbo's team has come to the conclusion that all non-African modern humans carry a small percentage (1–4%) of Neanderthal heritage, while those of purely African descent do not. In terms of population movements, this suggests that the small group of migrants that left Africa around 80,000 years ago to become the ancestors of all modern non-African populations must have encountered the Neanderthals (who had left Africa some 100,000 years earlier) presumably somewhere in the Middle East and mixed with them before their paths diverged to the various continents where their descendants ended up.

The Neanderthal genome is also a useful tool for the study of modern human diversity and its medical implications, as it (backed up by the more remote chimpanzee genome) can be used as a reference to indicate which of the several variants found in certain loci is likely to be the ancestral version.

Moreover, the genome revolution even expands the range of finds that can be analysed and linked to the family tree of human evolution. "These techniques can also be applied to small fragments of bones that one cannot know what kind of human they come from. We have done this for the first time using a finger bone from southern Siberia and shown that this bone comes from an individual that belonged to a population that shared an ancestor with Neanderthals but had a long independent history, which for example involved genetic exchange with the ancestors of people living in Papua New Guinea today," Pääbo explains. This work (J. Reich *et al.*, Nature (2010), *468*, 1053–1060) defined the new hominid group now known as the Denisovans – the first group of extinct hominids to be defined by genetic analysis rather than anatomical details.

Pääbo hopes that further discoveries will follow in the footsteps of the Denisovans. "We want to provide deeper sequence coverage of the Neanderthal genome, and also look for other human forms for example in Asia and Siberia. I believe that it will become possible to use DNA sequencing to reconstruct in quite some detail the population history of the different forms of archaic humans that existed until 30,000 to 50,000 years ago in Europe and Asia, and perhaps Africa."

However, the big prize, to him, is to understand what makes us human: "In the longer run, it is even more fascinating to me that the Neanderthal genome allows us to determine what changed in the human genome in the very last stage of human evolution, when fully modern humans who develop the culture and technology that allowed them to spread across the whole globe and eventually to dominate much of the biosphere. This enormous development required, I believe, some genetic events. It would be wonderful to be able to find at least a few of them," Pääbo concludes.

Old bones gain new life

With all the excitement over the genomics, discovery of new bones and better characterisation of the ones already known is still an important part of palaeoanthropology, and this is also revolutionised by modern technology.

Jean-Jacques Hublin, the director of the department of human evolution at the Leipzig institute, has pioneered the use of medical imaging technology such as X-ray scans to create virtual representations of hominid fossils, which his team can then study in greater detail than might be possible with the valuable and fragile original specimens.

"Virtual palaeoanthropology allows operations such as virtual cleaning and reconstructions impossible on the real objects," Hublin explains. "More importantly, it provides a unique way to access internal structures and micro-structures in a non-destructive way. It also allows a fine quantitative assessment of anatomical structures as well as simulation of growth patterns or evolutionary changes."

Hublin has a mobile X-ray laboratory that allows him to take the technology to the locations around the globe where hominid treasures are kept. He has already scanned pieces in collections based in Israel, South Africa, Kenya, Morocco, Croatia, and Russia. However, he denies the impression created by a recent press report that he wants to create a complete digital archive. "We accumulate digital data on hominid fossils in the course of our research work. However, we are a research institution not a museum and our goal is not to built up a global virtual archive," Hublin clarifies.

For instance, in a recent paper analysing the brain development of Neanderthals and modern humans after birth, Hublin's group used virtual reconstructions of Neanderthal skulls for their analyses (Curr. Biol. (2010), *20*, R921–R922). The researchers found that the characteristically different skull shape — more globular in adult humans, more elongated in Neanderthals — is due to a divergent development in the phase immediately after birth, when the skull is still malleable and responds directly to the growth pattern of the brain. It appears that the 'globularisation' phase after birth, which makes our braincase more spherical, is a unique, derived feature of human post-natal development.

Hublin's group also applied similar analysis to the development of the Neanderthal's characteristic facial features, including the size of the face in comparison to the braincase. The analyses confirmed earlier reports suggesting that these differences are established before birth. In a separate effort, Todd Rae from Roehampton University in London, UK, and others have used CT scans of Neanderthal skulls to test earlier hypotheses that the face was shaped by adaptation to cold climate. Analysing the sizes of the sinuses of nine Neanderthal individuals compared to 26 modern humans from Lithuania, Rae *et al.* conclude that Neanderthals' faces weren't particularly adapted to cold (J. Hum. Evol. (2011), *60*, 234–239).

Beyond the analysis of the human remains, modern imaging technology also helps the analysis of the tools that early modern humans and Neanderthals produced. In some cases, the two interests overlap. Christine Verna from the Leipzig institute and Francesco D'Errico from Bordeaux University have recently reported detailed analysis of Neanderthal bone fragments from the La Quina site in Southwest France, which they believe to be the earliest example of bone tools created by humans from human material (J. Hum. Evol. (2011), *60*, 145).

Unlike Hublin, the NESPOS (Pleistocene People and Places) project, launched and financed by the foundation that runs the Neanderthal Museum at the Neander valley site near Düsseldorf, Germany, and by the EU project TNT (The Neanderthal Tools), does aim to build a virtual archive of all the electronic information available on Neanderthals and their contemporaries. The TNT project started with 600 digital scans of Pleistocene remains and tools from across Europe. Researchers are now continuously adding to the NESPOS database, which is accessible on a subscription basis (nespos.org).

As for the future of the field that is now being modernised so rapidly, finding fossils will remain crucial. "There are still new hominids to be found. Our maps and evolutionary trees are full of blank zones," Hublin says. However, what scientists can do with the bones that they have is already futuristic to those who have experienced research in the 20th century. "Geochemistry and biochemistry of the fossil tissues unveil a wealth of information on the biology and behaviour of extinct humans. Dealing with their genes, diet, mobility, or life history the way we do today was simply beyond imagination when I was a student," Hublin concludes.

Old technology: Researchers and volunteer helpers sift through miners' rubble outside the cave of Sima de las Palomas in the Murcia district, southern Spain. Systematic excavations inside the cave have recently yielded three articulated skeletons of Neanderthals who were apparently buried there. (Photo: M. Gross.)

Keep digging

However, even the most advanced technology cannot replace the hard graft required to find fossilised remains. For instance, at the Sima de las Palomas site in the Murcia district, Southern Spain, researchers and scores of volunteer helpers have spent many summers excavating, following the discovery of a crushed facial skeleton in 1991, finding occasional bone and tooth fragments. Only in recent years their persistence was rewarded with the discovery of three articulated Neanderthal skeletons, including two adults and one adolescent (Proc. Natl. Acad. Sci USA (2011), *108*, 10087–10091).

Michael Walker from the University of Murcia, who leads the excavation at this site, is also applying the most modern technology available to the old skeletons. "We have a new scanner here at our Veterinary Faculty that is excellent for our work," Walker explains. "Using very complicated computer programs we can see where bones exactly lie within the masses of cemented matrix excavated at the site, and thanks to these programs in my lab we are painstakingly uncovering and cleaning the bone using vibroincisors driven by compressed air that pulverize the calcium-carbonate concretions covering the bones at 33,000 pulsations per second; this also is state-of-the-art technology that would have been unthinkable twenty years ago when we started excavating at the site." For scanning and reconstruction work, Walker also collaborates with the husband-and-wife team of Christoph Zollikofer and Marcia Ponce León at the University of Zurich.

Many more exciting ancient relics surely await to be unearthed and to be investigated in unprecedented detail thanks to new technologies.

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